PATENT
Docket No. 140942000401

#12/Ref
05-13-03

CERTIFICATE OF MAILING BY "FIRST CLASS MAIL"

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on April 28, 2003.

Tami M. Procopio
~~Marian L. Christopher~~ Tami M. Procopio

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Erin E. MURPHY, *et al.*

Serial No.: 09/840,795

Filing Date: April 23, 2001

For: MAMMALIAN GENES; RELATED REAGENTS

Examiner: Eileen B. O'Hara

Group Art Unit: 1646

DECLARATION OF JEANINE D. MATTSON
PURSUANT TO 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Jeanine D. Mattson, declare as follows:

1. I am a co-inventor of the above-referenced patent application, and am familiar with the contents thereof.

2. I have a Master of Arts in Cell and Molecular Biology in 1992 from San Francisco State University, awarded in 1992, and a Bachelor of Science in Biology from University of California, Riverside, awarded in 1984. I am currently a Senior Scientist at the DNAX Research Institute and have worked with DNA since 1986. I have expertise in DNA library construction, hybridization, Southern blot analysis, isolation of total and polyA RNA, and PCR. A full curriculum vitae is attached as Exhibit D.

3. I have conducted experiments demonstrating that SEQ ID NO:17 was expressed in the lung during inflammation-mediated responses and/or diseases, as disclosed in the instant specification. The experimental results are set forth in the following paragraphs 3-6.

3. *Gene expression analysis.* Real time PCR analysis was employed to quantify gene expression. Briefly, mRNA was isolated using conventional methodology. The Taqman® system (Roche Molecular Systems) was then employed to quantify gene expression using the sequence of SEQ ID NO:17 per manufacturer's instructions. The three C-terminal amino acids were not included in the SEQ ID NO. 17 used in the Taqman® analysis. Using ubiquitin mRNA as the standard (or baseline) activity, relative increases in FRET expression were determined between various samples. The expression profile for the truncated SEQ ID NO:17 was determined using mRNA preparations from human tonsil, lung, colon, thyroid, and skin, as well as *C. macaque* lungs. *C. macaques*, a primate model, represented a recognized animal model for human disease. Samples included normal and diseased tissue. Of significance, idiopathic pulmonary fibrosis was a disease resulting from alveolar inflammation that resulted in fibrosis and interstitial pneumonia. Similarly, an *Ascaris* challenge in a *C. macaque* also resulted in alveolar inflammation.

4. In Experiment #1, the expression of truncated SEQ ID NO:17 was increased exclusively in lung undergoing an inflammatory reaction or disease state. As shown in Table 1, detectable SEQ ID NO:17 expression was not observed in control human lung or control *C. macaque* lung. However, lung with idiopathic pulmonary fibrosis showed a greater than 20 fold increase in expression relative to the control lung. Likewise, in the *C. macaque* samples, lung samples taken 24 hours post-challenge with *Ascaris* had a greater than 20 fold increase in SEQ ID NO:17 expression.

5. Experiment #2 also showed the expression of truncated SEQ ID NO:17 was increased during an inflammatory reaction or disease state. *See* Table 2. As seen in the previous experiment, truncated SEQ ID NO:17 expression was increased 24 hours post-*Ascaris* challenge in the *C. macaque* lung.

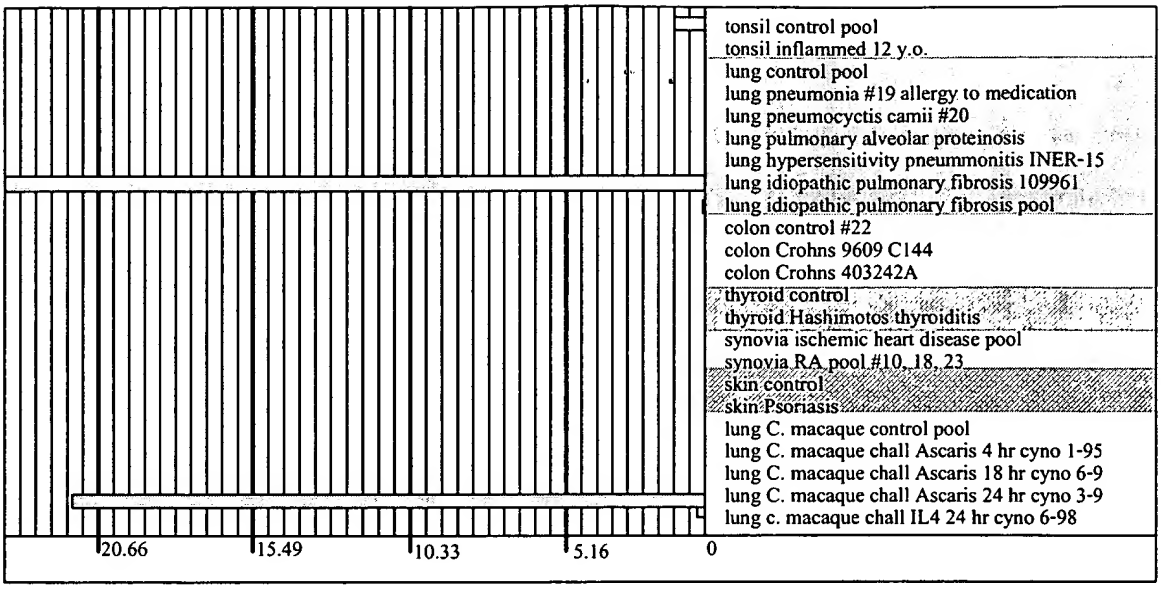
6. Taken together, these experiments demonstrated preferential expression of SEQ ID NO:17 in the lung during inflammation-mediated responses and/or diseases, as disclosed in the instant specification.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Executed at Palo Alto on April 25th, 2003.

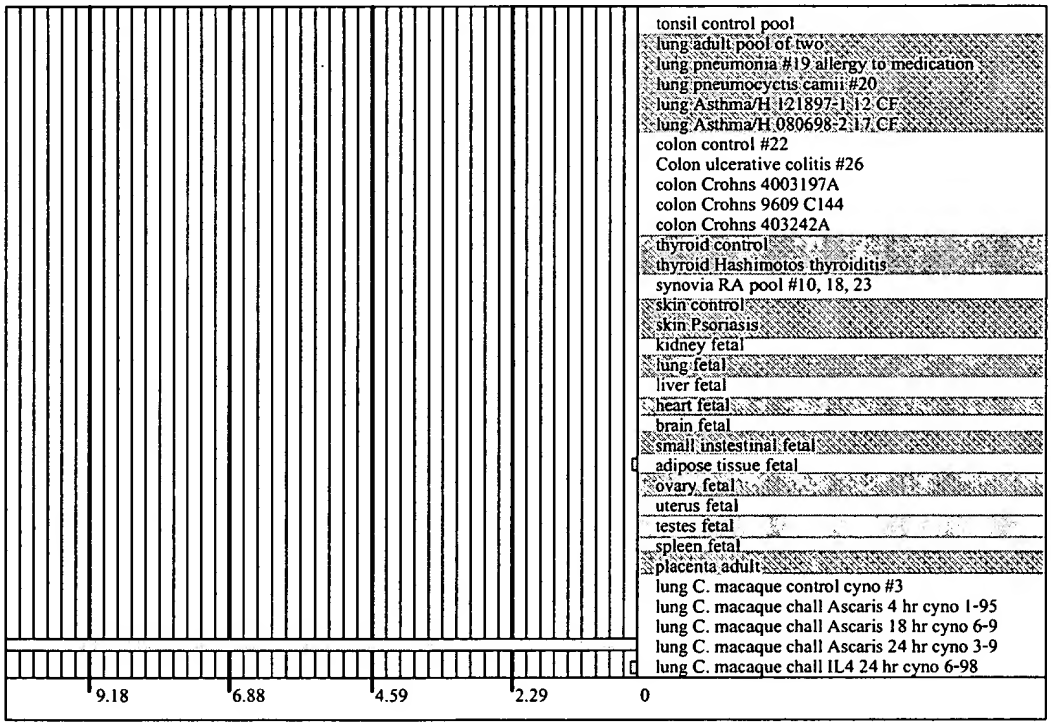
Jeanine D. Mattson

Table 1



Decrease Scale	Scale: Fold difference relative to Ubiquitin Sample Size:20.0 ng	Increase Scale

Table 2



Decrease Scale	Scale: Fold difference relative to Ubiquitin Sample Size:20.0 ng	Increase Scale

JEANINE D. MATTSON

EDUCATION: Master of Arts, Cell and Molecular Biology, January 1992
San Francisco State University

Bachelor of Science, Biology, May 1984
University of California, Riverside

EXPERIENCE: Senior Scientist
DNAX Research Institute

October 2000 to present

Research Associate/ Scientist II

January 1998 to October 2000
Designed taqman primers for entire gene familes and assessed reagents for specificity and efficiency. Determined expression distribution of novel and known genes on cDNA library panels and biological expression readouts of mouse disease models using Taqman analysis. Isolated same and cross species (Human, mouse, guinea pig,dog and monkey), full length clones of novel genes by nested PCR on cDNA libraries, cDNA and genomic DNA. Supervised junior technician.

Scientist III

July 1994 to January 1998
Responsible for constructing subtraction hybridization, RDA, plasmid and Lambda Ziplox cDNA libraries. Isolated same and cross species (Human, mouse, guinea pig, and monkey), full length clones of novel genes by colony hybridization screening and nested PCR on cDNA libraries. Organized, coordinated and generated PCR fragments of mouse and human clones for microarray analysis. Determined expression distribution of novel genes by Southern blot and Taqman analysis of cDNA library panels. Determined identity and homology of novel clones by use of bioinformatic programs with public/private databases. Provide patent lawyer with necessary biological information for application purposes. Wrote protocols for in-house distribution. Supervised junior technician.

Scientist III

August 1992 - July 1994
Responsible for cloning known genes and constructing gene fusions for in-house use in expression vectors. Created recombinant Baculovirus of desired genes for expression purposes and responsible for small-scale protein expression with BEV system. Small scale protein purification of tagged proteins using anti-Flag M2 antibody gel (Kodak) and Sepharose CL-4B gel for Ig G fusion's (Pharmacia). Western blot analysis of purified products.

Scientist II
Sandoz Agro, Inc.

October 1989 to July 1992
Isolated and cloned natural toxin genes by PCR of cDNA. Constructed baculo-virus expression vectors containing synthetic neuro- peptides and toxins, and natural toxin genes. Screened putative recombinant viruses by Southern blot and PCR for proper insertion of the desired genes. Isolated

polyhedra. Prepared viral DNA from occluded and nonoccluded virus. Lipofectin and calcium chloride transfections of Spodoptera frugiperda (fall armyworm) cell lines with Autographa californica (AcNPV; alfalfa looper virus) baculovirus and AcNPV baculovirus transfer vectors containing promoter - reporter gene (CAT) cassettes. Prepared frozen competent cells for general use. Compiled data on all vector constructs in the department on Microsoft Excel and IBM clones program. Organized in-house biweekly seminars. Techniques employed were polymerase chain reaction (PCR), Southern blot, colony and dot blot hybridization, nick translation, end- and random prime labeling, and in vivo excision of cDNA.

Research Associate II
Gladstone Foundation Laboratories for
Cardiovascular Research

September 1988 - September 1989
Identified cis-acting regulatory sequences of the 5' upstream region of the Human Apolipoprotein B gene by gel retention and DNAseI footprinting assays. Prepared nuclear protein extracts from mammalian cell lines of hepatic (HepG2jgs), intestinal (CaCo-2), and epithelial (HeLa) origin and maintained the cell lines. Isolated and radioactively end-labeled (5' or 3') DNA fragments for gel retention and DNAseI footprinting assays. Purified oligonucleotides by Native PAGE and oligonucleotide purification cartridges. Performed radioactive wipe tests and maintained disposal records.

Thesis Research
Genencor, Inc.

October 1986 - May 1988
Purified Bacillus subtilis' Bacillopeptidase F (BpF) by ultrafiltration/difiltration and DEAE Tris-acryl M column chromatography. Characterized the Bacillopeptidase F protein by determining its molecular weight, antigenic cross-reactivity, presence or absence of carbohydrate, isoelectric point, and amino acid compostion. Techniques employed were SDS PAGE, Native PAGE, FPLC, Immunodiffusion, Phenol-Sulfuric acid assay, Isoelectric focusing, and Acid hydrolysis. Obtained a partial clone of the BpF gene. Techniques employed were Genomic library construction, Southern blot, Colony hybridization, end-labeling, and Dideoxy DNA sequencing.

Part-time Research Assistant
Genencor, Inc.

June 1986 - July 1987
Isolated, purified and examined the physiological characteristics of soil microorganisms that exhibited specific hydrocarbon metabolism. Prepared summary protocols of experiments. Assisted in establishing an offsite labaratory for coordinated research. Compiled research data in a Lotus 123 program designed to screen data by single or multiple parameters.

Laboratory Assistant
Department of Nematology
University of California, Riverside

November 1984 - June 1985
Initiated and maintained bacterial-feeding nematode (genus Acrobeloides) and Aphelenchus avenae cultures. Performed anhydrobiotic experiments

on the nematode genus Acrobeloides by use of glycerin-water chambers, sulfuric acid chambers and soil moisture pressure plates. Determined optimum temperatures for bacterial-feeding nematode by observing total egg production and nematode populations at different temperatures (21 ^{o}C-18 ^{o}C) on bacterial-feeding nematode egg development. Performed monoxenic food source experiments with bacterial feeding nematode. Studied the ecological interactions of Meloidogyne incognita, Escherichia coli, and Acrobeloides (species unknown) and their effects on Tropical tomato plants. Prepared media.

June 1983 - November 1984
Assisted Ph.D. candidate with laboratory and field research used for dissertation "The Effects of Soil Bacteria on Meloidogyne incognita (Kofoid and White) on Chitwood infection". Prepared materials, performed in vitro and in vivo experiments, maintained bacterial and actinomycete stock cultures, collected data, and performed statistical data analysis on teletypes in Basic language. Mini-project on control of nematode, Meloidogyne incognita , by use of biological control agent (peptone) versus pesticide treatments (Temik and Vydate) on Tropical tomato plants.

October 1981 - June 1983
Prepared media, inoculated plants with nematodes, and isolated nematodes from soil by Baermann funnel process.

TECHNIQUES: cDNA library construction, subtraction library construction, size selected library construction, Colony hybridization for same and cross species full length clones, Dot blot hybridization, Southern blot analysis, Isolation of total and polyA RNA, PCR.

SKILLS: Familiar with use of bioinformatic systems for novel gene discovery: blastN, X and P, Sequencher™, Macvector™ and Vector NTI7™. Familiar with ABI taqman software, Primer Express™ and SDS 2.1™

THESIS: "Purification and Cahracterization of Bacillus subtilis' Bacillopeptidase F (BpF) Protein and Partial Cloning of the BpF gene."

PUBLICATIONS: Pflanz, S.; Timans, J.C.; Cheung, J.; Rosales, R.; Kanzler, H.; Gilbert, J.; Vaisberg, E.; Blumenschein, W.; Mattson, J.D.; Wagner, J.; To, W.; Zurawski, S.; McClanahan, T.; Gorman, D.M.; Bazan, J.F.; de Waal Malefyt, R.; Rennick, D. and Kastelein, R.A. 2001. "IL-26, a heterodimeric cytokine composed of EBI3 and novel p28, is a clonal expansion factor for naïve CD4+ T cells" Immunity 16:779-790.

Blom, B.; Ho, S.; Mattson, J.; Zurawski, S.; McClanahan, T.; Gorman, D.; Zurawski, G.; and Liu, Y.-J. 1998. "Two Newly Identified Members of the TNF Ligand Superfamily Specifically Expressed on Dendritic Cells Costimulate B cells". Abstract."5th International Symposium of Dendritic Cells in Fundamental and Clinical Immunology".

McClanahan, T. et al. 1996. "Biochemical and genetic characterization of multiple splice variants of the Flt3 ligand". Blood 88(9):3371-3382.

Phillips, J.H.; Chang, C.; Mattson, J.; Gumperz, J.E.; Parham, P.; and Lanier, L.L. 1996. "CD94 and a novel associated protein (94AP) form a NK cell receptor involved in the recognition of HLA-A, HLA-B and HLA-C allotypes". Immunity 5:163-172.

Leisy, D.J.; Mattson, J.D.; Quistad, G.B.; Kramer, S.J., van Beek, N.; Tsai, L.W.;

Enderlin, F.E.; Woodworth, A.R. and Digan, M.E. 1995. "Molecular cloning and sequencing of cDNAs encoding insecticidal peptides from the Primitive Hunting Spider, *Plectreurys tristis* (Simon)" Insect Biochem. Molec. Biol. 26(5):411-417.

Murphy, E.E. et al.1994. "B7 and Interleukin 12 cooperate for proliferation and Interferon γ production by mouse T helper clones that are unresponsive to B7 costimulation". J. Exp. Med. 180:223-231.

Hannum, C. et al. 1994. "Ligand for FLT3/FLK2 receptor tyrosine kinase regulates growth of haematopoietic stem cells and is encoded by variant RNAs". Nature368:643-648.

PATENTS:

Mattson,J.D.; McClanahan, T. 2003 U.S. Patent application "Canine RANKL And Methods For Preparing and using Same. [Filed]

Basham, B.E.; Gorman, D.M.; Mattson, J.D.; Moshrefi, M. 2002 U.S. Patent application "Mammalian Genes; Related Reagents" [C1QSF] Filed

Basham, B.E.; Forsythe, I.; Gorman, D.M.; Mattson, J.D.; Moshrefi, M. and Parham, C. 2001. U.S. Patent application "Mammalian Genes; Related Reagents" [C1QSF] Filed.

Gorman, D.M. and Mattson, J.D. 2000.. U.S. Patent application "Mammalian Cell Surface Antigens; Related Reagents". [Rank ligand] Granted.

Parham, C.L.; Gorman, D.M; Kurata, H.; Arai,N.; Sana,T.; Mattson, J.D.; Murphy, E.E.; Savkoor,C.R.; Grein. J.; Smith, K.L. and McClanahan, T.K. 2000. U.S. Patent application "Mammalain Genes; Related Reagents and Methods". [Schlafen] Filed.

Deng, G.; Catron, D.J.; Mattson, J.D.; de Waal Malefyt, R.; McClanahan, T.K.; Zlotnik, A.; and Coffman, R.L. 1999. U.S. Patent application "Th2 cell depeletion; Compositions; Methods". [STRL33] Filed.

Bates, E.E.M.; Lebeque, S.J.E. ; Murphy, E.E.; Mattson, J.D.; Gorman, D.M ; Hedrick, J.; Wang, L.; Zlotnik, A. ; Murgolo, N.; Greene, J.; Johnston, J.A.; Bazan, J.F.; Mahoney, D. and lees, E.1999. U.S. Patent application " Mammalian Genes; Related reagents". [HDTEA84/XEDAR] Published.

Mattson, J.D.; McClanahan, T.K. and Kastelein, R.A. 1998. U.S. Patent application "Human Receptors; Related reagents and Methods". [DCRS1] Inactive.

Mattson, J.D.; Soto-Trejo, H.; Hedrick, J.A.; Gorman, D.M. and Zlotnik, A. 1998. U.S. Patent application "Mammalian chemokines; Receptors; Reagents; Uses". [Hu GPCR] Published.

REFERENCES: Available upon request

9 Infectious and Parasitic Diseases

Daniel H. Connor and Dean W. Gibson

Figure 9-1. Epidemiology of yellow fever. The usual reservoir for the yellow fever virus is the tree-dwelling monkey. The virus is passed from monkey to monkey in the forest canopy by mosquitoes of the genus *Aedes*. Felling a tree brings mosquitoes down with the tree, increasing the chance of being bitten and inoculated with the virus.

cause minute colonic ulcers and mild inflammation. Rare but serious complications arise when female worms migrate through the vagina, uterus, and fallopian tube, and reach the peritoneum and omentum. In these extraintestinal sites the worms die and provoke a granulomatous reaction.

The diagnosis is made by identifying eggs in the perianal area, using various swab techniques. Eggs are not usually found in feces. Antihelminthic drugs are effective.

Ascariasis

Ascariasis, infection by the large intestinal roundworm *Ascaris lumbricoides*, is cosmopolitan and probably **the most common helminthic infection.** Infection is more common in the tropics, in children, and in crowded rural communities with poor sanitation. Adult worms live in the small intestine, where gravid females discharge eggs that pass in the feces. Eggs develop in warm moist soil to become infective in 3 to 4 weeks. The eggs hatch when ingested, and the larvae penetrate the intestinal wall, enter the portal circulation, pass through the liver and heart, reach the lungs, and develop into third-stage larvae. The larvae migrate up the trachea and down the esophagus, reaching the small intestine, where they develop into adult worms. Humans acquire the infection by ingesting infective eggs in contaminated soil, food, or water.

Figure 9-78. Ascariasis. This mass of over 800 worms *Ascaris lumbricoides* obstructed and infarcted the ileum of a 2-year-old girl in South Africa who died before she could be treated.

Most patients have few or no symptoms. Infection with a few adult worms causes only vague abdominal pain, but heavy infections may cause vomiting, malnutrition, appendicitis, and sometimes intestinal obstruction (Fig. 9-78).

Adult worms in the small intestine usually cause no changes, but when worms migrate into the ampulla of Vater or the pancreatic or biliary ducts, they cause biliary obstruction, acute pancreatitis, suppurative cholangitis, and liver abscesses. Eggs deposited in the liver or other tissues produce necrosis, granulomatous inflammation, and fibrosis. *Ascaris* pneumonia, which is occasionally fatal, develops when larvae migrate within alveolar walls, air sacs, bronchioles, and bronchi. The exudate is composed of eosinophils, macrophages, and fibrin.

The diagnosis of ascariasis is made by identifying eggs in the feces. Occasionally, adult worms may pass with the stool or even emerge from nose or mouth. Ascaricidal drugs are effective.

Anisakiasis

Anisakiasis is infection by ascarid larvae of the genera *Anisakis, Phocanema, Terranova,* and *Contracaecum.* Aniskiasis is contracted when inadequately cooked fish containing these nematode larvae are eaten. Popular foods containing viable larvae are pickled herring in Scandinavian countries, sashimi in Japan, and cod, flounder, and tuna from the east coast of the United States. Nematodes causing anisakiasis have a marine mammal as the definitive host.

In humans the larvae penetrate the wall of the throat, stomach, intestine, or colon. Intestinal discomfort begins a few hours after eating the fish. Larvae are released from the muscle of the fish, penetrate the gastric or intestinal mucosa, or become attached in the throat without invasion of tissue. Infection is diagnosed after surgical intervention for intestinal obstruction or peritonitis, which is caused by necrotizing, eosinophilic, granulomatous inflammation. Worms in the throat or stomach are frequently vomited or coughed up, and a common clinical presentation is a wriggling sensation in the throat, with larvae appearing in the mouth of an alarmed patient.

Intestinal anisakiasis clinically resembles appendicitis, with right lower quadrant pain, nausea and vomiting. Continued migration to omentum or mes-

Pathology

Edited by

Emanuel Rubin, M.D.

Gonzalo E. Aponte Professor and Chairman
Department of Pathology
Jefferson Medical College
Thomas Jefferson University
Philadelphia, Pennsylvania

John L. Farber, M.D.

Professor of Pathology
Jefferson Medical College
Thomas Jefferson University
Philadelphia, Pennsylvania

With 40 contributors

Illustrations by
Dimitri Karetnikov

J. B. Lippincott Company
Philadelphia
London Mexico City New York
St. Louis São Paulo Sydney

12

The Respiratory System

William M. Thurlbeck and Roberta R. Miller

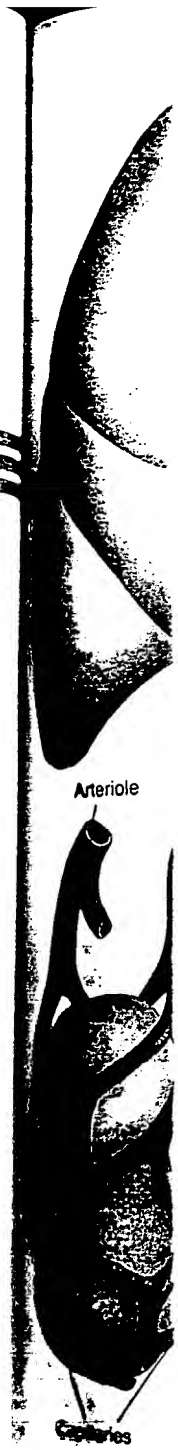


Figure 12-1. Anatomy of the lung. The conducting structures of the lung include (1) the trachea, which has horseshoe-shaped cartilages; (2) the bronchi, which have plates of cartilage in their walls (both the trachea and bronchi have mucus-secreting glands in their wall); and (3) the bronchioles, which do not have cartilage in their walls. The most distal is the terminal bronchiole. The gas-exchanging components comprise the unit distal to the terminal bronchiole, namely, the acinus. Alveoli are lined by type I cells, which are large, flat cells that cover most of the alveolar wall, and by type II cells, which secrete surfactant and are the progenitor cells of the alveolar epithelium. The alveolar capillaries exchange gas in the alveolar wall.

irregular. Extensive vascular changes, particularly intimal fibrosis and thickening of the media, are caused by inflammation, fibrosis, and pulmonary hypertension.

Usual Interstitial Pneumonia (Cryptogenic Fibrosing Alveolitis, Idiopathic Pulmonary Fibrosis)

Each of the terms *usual interstitial pneumonia, cryptogenic fibrosing alveolitis,* and *idiopathic pulmonary fibrosis* stresses certain features—the cause is unknown, alveolar inflammation is an important part of the disease, fibrosis is the usual sequel, and there may be unusual forms of interstitial pneumonia. The clinical, radiologic, and functional features are those of restrictive lung disease (Fig. 12-39). A useful classification of usual interstitial pneumonia is as follows, but it should be recognized that the morphologic features are the same in each:

- **Usual interstitial pneumonia associated with collagen vascular diseases.** These include rheumatoid arthritis, systemic lupus erythematosis, and progressive systemic sclerosis. About 20% of cases of usual interstitial pneumonia have overt evidence of a connective tissue disease.
- **Usual interstitial pneumonia associated with serum abnormalities but not with collagen vascular disease.** These include cryoglobulinemia, abnormal serum globulins, positive antinuclear antibodies, and positive rheumatoid factor (rarely positive in the absence of rheumatoid arthritis). These abnormalities have been found in up to 40% of cases.
- **Usual interstitial pneumonia without overt evidence of collagen vascular disease or serum abnormalities.**

The key morphologic feature of usual interstitial pneumonia is heterogeneity of lesions, that is, different appearances in different parts of the lung, in different lung biopsies, and even in different fields of the same lung biopsy. The variation is so great that in some fields the alveolar walls are entirely normal. Inflammation varies from subtle increased cellularity (mainly lymphocytes) of otherwise apparently normal alveolar walls to diffuse alveolar damage with obvious alveolar wall inflammation and hyperplasia of type II cells. Lymphoid aggregates are also seen. By the time a lung biopsy is performed fibrosis is always present, but its severity varies. There may be subtle alveolar wall thickening, detectable only by special stains for collagen. In other c fibrosis may be obvious but alveolar walls ma maintained, although the acinar structure is sc what simplified. At the extreme is honeycomb l brought about by alveolar wall inflammation and lapse. This condition is characteristically most pr inent subpleurally in the lower zones of the lung 12-39). Electron microscopic examination re gross distortion and infolding of the alveolar l lamina in the fibrotic areas. Loose granulation ti in the alveolar spaces leads to alveolar collapse contraction of fibrous tissue.

Interstitial pneumonia is usually diagnosed in the sixth decade. Dyspnea of gradual onset, often over 5 to 10 years, is customary. About one quarter of all cases date their illness to an acute bronchopulmonary infection, an observation that raises the possibility that the disease is a sequel to viral infection. The classic auscultatory finding consists of fine crackles at the lung bases in late inspiration. The prognosis is bleak, with an average survival of 5 years. The response to treatment, usually corticosteroids, is generally poor.

The etiology of usual interstitial pneumonia is not known, but the condition is usually thought of as an immunologically mediated disorder. Evidence includes the association with collagen-vascular disease and serum protein abnormalities, the presence of circulating immune complexes, the presence of immunoglobulins in alveolar walls, and the release of a lymphokine, migration inhibitory factor, when lymphocytes of patients with the disease are exposed to collagen.

According to one theory, macrophages play a central role in the pathogenesis of usual interstitial pneumonia, and the initial damage is to collagen, by an unknown agent. Macrophages engulf collagen fragments and secrete a fibroblast-stimulating factor, thereby leading to fibrosis. They also release a chemotactic factor for polymorphonuclear leukocytes, which then do further damage. Histologically, neutrophils are not a prominent feature, except in the infected cystic spaces. However, the bronchoalveolar lavage fluid contains increased numbers of neutrophils.

Desquamative Interstitial Pneumonia

Desquamative interstitial pneumonia (DIP) is an uncommon disease characterized by interstitial inflammation and a striking accumulation of macrophages in the alveoli. Opinion is equally divided on whether it is a separate entity or a stage of usual interstitial pneumonia. However, it is important to recognize